Minireview

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Purine Phosphoribosyltransferases*

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Purine phosphoribosyltransferases (PRTs)[1] of microbes and mammals are enzymes that catalyze the recovery of preformed bases for use in cellular metabolism. In free living organisms, purine nucleotides can be generated via *de novo* synthesis, as well as by the salvage of preformed bases. In contrast, many parasitic organisms are unable to synthesize purines via *de novo* pathways and therefore must rely on the enzymes in salvage pathways, including PRTs, for the synthesis of purine nucleotides (1). For this reason, enzymes in salvage pathways were proposed more than 30 years ago as potential targets of therapeutic agents for the treatment of diseases caused by parasites (2).

Purine PRTs catalyze the reversible transfer of a phosphoribosyl group from phosphoribosylpyrophosphate (PRPP) to a purine base (adenine, guanine, hypoxanthine, or xanthine). For those enzymes that have been studied, the forward reaction appears to be ordered and sequential with PRPP binding first followed by the purine base (3–5). After catalysis, pyrophosphate (PP_i) is released before the nucleotide. Reaction chemistry has been reported to proceed via either a dissociative (S_n1) or an associative (S_n2) type mechanism (Fig. 1). Based on their similarity to other enzymes, PRTs have been proposed to catalyze an S_n1-type reaction with the formation of an unstable ribooxocarbenium ion intermediate (6, 7), but S_n2-type chemistry for purine PRTs has not been ruled out (8).

Enzymes in salvage pathways that contribute to the synthesis of AMP include adenosine kinase or adenine phosphoribosyltransferase (APRT). Alternatively, adenosine deaminase and a purine nucleoside phosphorylase catalyze the conversion of adenine to hypoxanthine, which may be salvaged by hypoxanthine phosphoribosyltransferase (HPRT). The product, IMP, is a precursor for both AMP and GMP. In most bacteria and nearly all eukaryotes, HPRTs also catalyze the salvage of guanine and, in a few cases, xanthine. In human tissues, substrates available for salvage include hypoxanthine at 8.2 ± 1.3 μM, xanthine at 2.5 ± 0.6 μM, adenine at 0.3 ± 0.15 μM, and adenosine at 0.6 ± 0.2 μM (9). The human HPRT can salvage xanthine, albeit at relatively low levels (10), but instead this base is usually converted to uric acid for excretion as a nitrogenous waste. The identity of the 6-oxopurine substrates salvaged by HPRTs is frequently included in the names of these enzymes, but HPRTs likely are descended from a common ancestral *hpt* gene of prokaryotes, and substrate specificity can be dramatically altered by single amino acid substitutions (11, 12).

Several bacteria possess two distinct enzymes for the salvage of 6-oxopurines. In those bacteria the primary substrate for the HPRT is hypoxanthine, and guanine is utilized with reduced efficiency (11). The other enzyme, referred to here as xanthine phosphoribosyltransferase (XPRT), has a preference for catalyzing the salvage of guanine and xanthine.

The contributions of specific enzymes to the salvage of purine bases vary significantly among different organisms. For example, in *Plasmodium falciparum*, etiologic agent of the most lethal form of human malaria, the activity of adenosine kinase is barely detectable, and APRT activity is 1500-fold below that of HPRT (13). In this pathogen, adenosine deaminase and purine nucleoside phosphorylase are relatively abundant, suggesting that the major route for the salvage of purine bases leading to both AMP and GMP is through hypoxanthine, which can be converted to IMP by the HPRT-catalyzed reaction.

Crystal Structures of Purine PRTs

For this review, residue numbers refer to the positions of amino acids in the human HPRT, the bacterial XPRT, or the leishmanial APRT, as these enzymes were the first of their class for which crystal structures were reported (14–16). Fig. 2 illustrates ribbon diagrams of monomers from these representative structures, although the enzymes may be functional as dimers or tetramers. All three of these enzymes possess a core domain composed of a 4- or 5-stranded parallel β sheet flanked by 3–4 α helices. The C-terminal ends of the β sheets of the core domains of purine PRTs form the floor of the active sites of these enzymes. A poorly conserved hood domain contributes residues that complete the active site and participate in binding purine substrates. For HPRTs and XPRTs the amino acids that flank the active site are contributed largely by 4 active site loops (loops I–IV). Residues in the hood domains of HPRTs, XPRTs, and APRTs come from non-homologous regions of the protein. However, all three enzymes possess an aromatic residue that forms π-π stacking interactions with purine substrates (Fig. 3).

Functional Roles for Conserved Amino Acids

Data reported to GenBank™ indicate that the HPRTs of distantly related organisms share extensive primary sequence homology. For example, there is 41% identity for amino acids in the human and a bacterial HPRT (11). However, among well over 20 HPRT sequences reported there are only 9 invariant amino acids and all but the HPRT of *Giardia lamblia* also are invariant at Glu-133 and Asp-134 (Table I). In general, conserved residues of HPRTs and bacterial XPRTs differ at positions homologous with human Leu-67 and Glu-133 (Ser-36 and Asp-88 in the XPRT of *Escherichia coli*). Solutions for the crystal structures of HPRTs reveal that the 11 conserved residues immediately flank or are very near the active site of HPRTs (Fig. 3).

Among the amino acid sequences deduced from 39 APRT genes reported to GenBank™, 11 residues are invariant. As shown in Fig. 3, these residues either flank the active site or are located within active site loop II, which is predicted to participate in forming the active site of APRTs during catalysis (16).

If the crystal structures of all purine PRTs are analyzed together with the amino acid sequences reported to GenBank™, there are only 2 residues (corresponding with human Gly-69 and Asp-134) that are clearly invariant. A G69E mutation virtually inactivates the human HPRT, resulting in Lesch-Nyhan syndrome, whereas a D134G mutation partially inactivates the enzyme, resulting in gouty arthritis (17). The invariant glycine may be essential for the formation of a tight turn and an unusual non-proline *cis*-peptide in active site loop I of purine PRTs (15, 16, 18–23).

In nearly all purine PRTs, there is a lysine or arginine residue located immediately upstream from the invariant glycine. There have been several suggestions for the functional role of the *cis*-peptide and the lysine (Lys-68) in active site loop I of HPRTs (20, 23, 24). The presence of the *cis*-peptide enables the carbonyl oxygen and amide nitrogen, adjacent to the peptide bond, to interact with pyrophosphate atoms and a metal-associated water molecule when they are present in the active sites of HPRTs (21–23, 25). Closed active site structures of HPRTs show that the side chain of Lys-68 forms multiple hydrogen bonds with residues in the opposing sub-

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[1] The abbreviations used are: PRT, purine phosphoribosyltransferase; PRPP, phosphoribosylpyrophosphate; APRT, adenine phosphoribosyltransferase; HPRT, hypoxanthine phosphoribosyltransferase; XPRT, xanthine phosphoribosyltransferase.

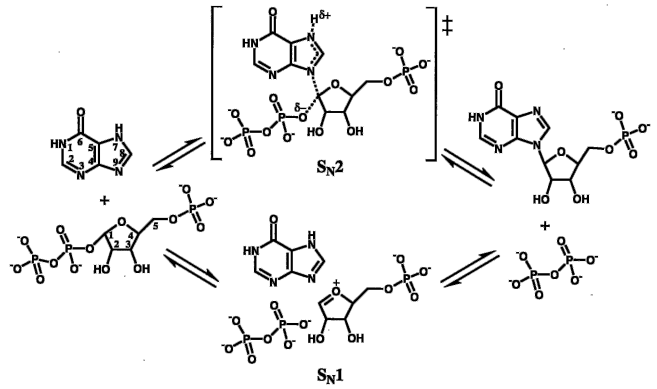

FIG. 1. **S_n1 *versus* S_n2-type reaction mechanisms catalyzed by hypoxanthine PRTs.** The ribooxocarbenium intermediate created in a two-step S_n1 mechanism is compared with the predicted transition state for an S_n2-type mechanism.

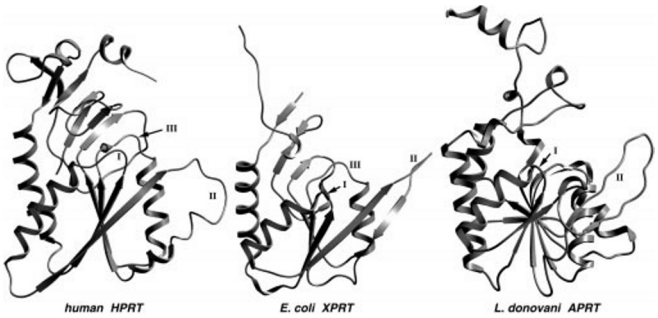

FIG. 2. **Ribbon diagrams of the human HPRT (14), the XPRT of *E. coli* (15), and the APRT of *Leishmania donovani* (16).** The similar orientations for the enzymes illustrate the conserved α/β structures of core domains below the non-conserved structures of hood domains. *Roman numerals* refer to active site loops I, II, and III.

unit in dimers of the enzyme (21, 22, 25). Changes in interactions involving Lys-68, before and after closure of the active site, as the enzyme approaches the transition state of the forward reaction coincide with the weakening or breaking of hydrogen bonds between main chain atoms of loop I residues and ligands in the active site (25). These interactions with active site ligands are replaced by new hydrogen bonds with main chain atoms of residues in active site loop II, which closes over the active site during the transition state. These changes in hydrogen bond interactions with substrates during closure of the active site may facilitate the liberation of pyrophosphate from the active site after catalysis. Directed mutation of Lys-68 to alanine in the human HPRT resulted in the observation of positive cooperativity between subunits in the binding of PRPP, a result that is consistent with loop I being involved in regulating binding interactions with PRPP and pyrophosphate (24). Furthermore, in the HPRT of *Tritrichomonas fetus* (a protozoan parasite of cattle), the threonine residue at position 68 was proposed to affect the binding of PRPP and PP_i to this enzyme (26).

All purine PRTs possess a dipeptide of acidic residues (human HPRT position 133–134 or leishmanial APRT position 146–147) within a conserved PRPP binding motif that spans 11 amino acids and includes residues of active site loop III. Among purine PRTs, the acidic dipeptide forms hydrogen bonds with the 2′- and 3′-hydroxyls of ribose moieties of substrates (16, 18, 19, 21, 22, 25, 27). Also, Glu-133 of HPRTs forms a hydrogen bond with a metal-associated water molecule when it is present in the active site.

A Catalytic Base

Among HPRTs, the aspartate at position 137 was proposed to play a role in catalysis by interacting with the N-7 position of 6-oxopurine substrates (21, 22, 28). Mutagenesis of the human HPRT indicated that this aspartate likely functions as a catalytic base by facilitating removal of a proton in forward reactions catalyzed by HPRTs (28). Also, hydrogen bonding between this aspartate and the purine N-7 position of a nucleotide analog may contribute to the very tight binding of this inhibitor to HPRTs (21, 22).

The residue at the analogous location in the crystal structure of

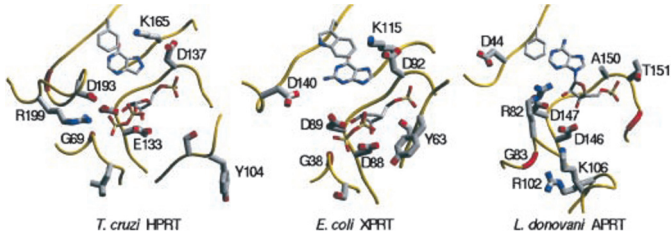

FIG. 3. **Conserved residues in the active sites of purine PRTs.** The enzyme from *Trypanosoma cruzi* is shown (numbered according to residues in the human HPRT) in ternary complex with a purine analog and PRPP (25). The bacterial XPRT is with guanine and a PRPP analog (19), and APRT has bound AMP (16). The main chain atoms of invariant glycines and side chains of other invariant amino acids are shown with thick bonds. Substrates, substrate analogs, and conserved aromatic side chains that form π-π stacking interactions with purine rings are illustrated with relatively thin bonds.

TABLE I

The highly conserved residues of HPRTs, XPRTs, and APRTs

Residues that may have analogous functions in all purine PRTs are printed in bold type. Although located at opposite ends of the protein sequences, Asp-193 of HPRTs, Asp-140 of bacterial XPRTs, and Asp-44 of APRTs are located at analogous positions in enzyme crystal structures and thus could possibly have similar functional roles (see text).

Human HPRT	Bacterial XPRT	Leishmanial APRT
		Phe-42
		Asp-44
Leu-67	Ser-36	Arg-82
Gly-69	**Gly-38**	**Gly-83**
Ser-103	Ser-62	Arg-102
Tyr-104	Tyr-63	Lys-106
Glu-133	**Asp-88**	**Asp-146**
Asp-134	**Asp-89**	**Asp-147**
Asp-137	Asp-92	Ala-150
Lys-165	Lys-115	Thr-151
Gly-189		Gly-153
Asp-193	***Asp-140***	
Arg-199		

the leishmanial APRT is a conserved alanine (Ala-150), suggesting that either the catalytic mechanism differs between HPRTs and APRTs or that the catalytic base of APRT is contributed by an amino acid that moves to participate in the formation of the active site during the transition state. In HPRTs, active site loop II has been shown to close over the active site when both substrate ligands are bound and presumably during the transition state of the reaction (21, 22, 25). Similarly, the x-ray crystal structure of the leishmanial APRT with bound AMP hints that residues within active site loop II of this enzyme may contribute to forming the active site during the transition state (16). Thus, a catalytic base might be contributed by a residue of active site loop II of APRTs.

A Site for Binding a Second Metal Ion

An aspartate at position 193 of HPRTs has been shown to participate indirectly in binding pyrophosphate and purines via the formation of a direct protein metal bond with a magnesium ion (21–23, 25) designated M2 (Fig. 4). Asp-193 also forms hydrogen bonds with two water molecules coordinated by the metal, and the metal forms coordinated interactions with two oxygens of PRPP or PP_i. A third coordinated water molecule forms another hydrogen bond with the N-3 atom of purine substrates. An invariant arginine at position 199 of HPRTs participates directly in binding pyrophosphate (29) and may contribute to positioning both substrates by being close enough to the carboxyl group of Asp-193 to affect its position (21–23, 25). Together, these interactions help to position both substrates for in-line nucleophilic attack at the C1′ carbon of PRPP or a nucleotide. A D193N mutation virtually inactivates the human enzyme resulting in Lesch-Nyhan's syndrome (17). An explanation for the devastating effects of this mutation is revealed by the existence of a hydrogen bond between the Asp-193 side chain and the main chain nitrogen of Asp-196 together with a preference of magnesium for interacting with oxygen over nitrogen. Thus, the substitution of asparagine for aspartate at position 193 would disfavor interactions with both Asp-196 and a magnesium ion.

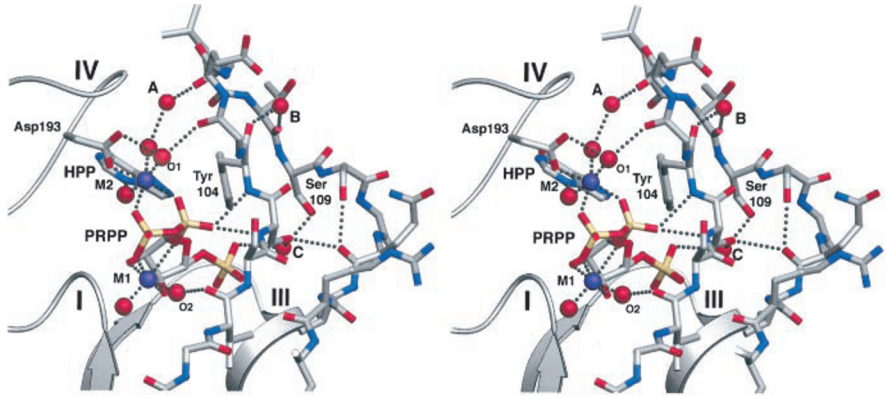

FIG. 4. **A cross-eyed stereo view of the closed active site of the HPRT from *Trypanosoma cruzi* in ternary complex with substrate ligands (25).** Interactions between active site ligands and amino acids in loop II are shown. Active site loops I, III, and IV are diagrammed as ribbons, and all non-hydrogen atoms of the closed loop II are illustrated. Water "C" is hydrogen bonded to the 5′-phosphate of PRPP as well as to the side chains of both Ser-103 (not labeled) and Ser-109 (see text). *HPP*, hydroxypyrazolopyrimidine. This figure has been reprinted with permission from *Biochemistry* (25). To view three-dimensionally, cross eyes slightly and focus on the central thrid image.

APRTs possess an invariant aspartate (Asp-44) that flanks the putative PP_i binding domain in the active site of the enzyme (Fig. 3). This suggests that a mechanism comparable with that of HPRTs and XPRTs, for binding a second metal and positioning substrates, could possibly exist for APRTs.

As of this writing, the only clear example in eucaryotes of multiple enzymes for the salvage of 6-oxopurines is in *Leishmania donovani* (30). In this protozoan parasite there is a gene encoding an "XPRT" as well as an HPRT. The two enzymes appear to be the product of a duplication of the *hpt* locus with both genes being expressed in extant parasites. The leishmanial XPRT is novel in its preference for catalyzing the salvage of xanthine over hypoxanthine and guanine. This enzyme differs from all other 6-oxopurine PRTs in possessing glutamate rather than aspartate at a position homologous with Asp-193 in the human HPRT. This substitution would be predicted to affect the positioning of the octahedrally coordinated second metal ion that participates in positioning purine substrates in the active site. Possibly, the D193E substitution in the *L. donovani* XPRT is responsible for the altered substrate specificity of this enzyme.

Roles for Residues in Active Site Loop II of HPRTs

As indicated in Table I, Ser-103 and Tyr-104 are invariant in HPRTs and XPRTs. Both are located in loop II, which closes over the active site during the transition state (Fig. 4). A S103R mutation has been shown to impair the human HPRT, resulting in gouty arthritis (17). The main chain carbonyl of Ser-103 forms a hydrogen bond with a pyrophosphate oxygen atom of PRPP, whereas the side chain hydroxyl interacts with a water molecule (Fig. 4, *C*). This water forms an additional hydrogen bond with a conserved serine (Ser-109), as well as to a 5′-phosphate oxygen of either PRPP or a nucleotide analog in the active site (21, 22, 25). These interactions secure the base of the closed loop II and stabilizes its position over the active site. Like the S103R mutation, a S109L mutation results in partial inactivation of the human HPRT (17). Steady-state kinetic studies of these mutations in the human HPRT (31, 32), together with contemporary structural data, indicate that loop II also contributes to the stable binding of purine substrates. Possibly this is achieved indirectly through van der Waals interactions of loop II residues with conserved aromatic residues (homologous with human Phe-186) that stack above purine substrates.

Analysis of the HPRT from *L. donovani* revealed that mutations of the residue homologous with the tyrosine at position 104 of the human HPRT severely reduce the turnover number (k_{cat}) for the forward reaction catalyzed by the enzyme (33). The main chain nitrogen of Tyr-104 forms a hydrogen bond with a pyrophosphate oxygen (Fig. 4), and the side chain hydroxyl forms a hydrogen bond with a 5′-phosphate oxygen atom of either PRPP or a nucleotide analog in the active site (21, 22, 25). These interactions position the aromatic ring of Tyr-104 directly above the location of the glycosidic bond that would be formed or broken during catalysis and nearly perpendicular to the ribose ring. Thus, the aromatic ring of tyrosine helps to isolate the reaction center from bulk solvent and could possibly provide partial electrostatic stabilization for a positively charged intermediate in an S_n1-type reaction.

Specificity for 6-Oxo- and 6-Aminopurine Substrates

An invariant lysine residue at position 165 in the human HPRT forms a hydrogen bond with the exocyclic oxygen of 6-oxopurine bases (14). The homologous residue in the HPRT of *T. fetus* has been demonstrated by site-directed mutagenesis to be the determinant for 6-oxopurine substrate specificity (12). In the only available structure for an APRT, specificity for 6-aminopurines appears to be determined by the formation of a hydrogen bond with a main chain carbonyl atom of an arginine residue at position 41 (16).

The Catalytic Mechanism of HPRTs

The discovery of two metal ions in the active sites of HPRTs has important implications for the chemistry of the reaction catalyzed by this enzyme. The electron withdrawing potential of the metal ions may contribute to activation of PP_i as a leaving group in HPRT-catalyzed reactions. The metal designated M1 forms no direct interactions with active site residues (Fig. 4). However, 4 oxygen atoms belonging to either PRPP or PP_i and the ribose moiety of nucleotide substrates (atoms designated O-1, O-2′, O-3′, O-3B) are within the coordination sphere of this metal (21, 22, 25). In the structure of the trypanosomal HPRT, with PRPP and a purine analog as ligands, the length of the coordinated interaction of M1 with the O-1 atom of PRPP is too long (at 2.6 Å) to satisfy optimally the metal coordination sphere (25). Thus, the electron-withdrawing potential of M1, along with formation of the sixth metal bond to O-1 as the reaction approaches the transition state, may contribute to lowering the activation energy for catalysis.

Previous kinetic studies (6, 7) suggest that HPRTs catalyze an S_n1-type chemical reaction (Fig. 1) where an unstable ribooxocarbenium intermediate would need to be protected from bulk solvent. The deletion of 7 residues from active site loop II of the trypanosomal HPRT does not prevent the enzyme from catalyzing either forward or reverse reactions, albeit at extremely reduced catalytic efficiencies (34). This indicates that total isolation from bulk solvent is not required for the protection of a highly unstable intermediate in the reaction. Furthermore, x-ray crystal structures of the closed active sites of the human, malarial, and trypanosomal HPRTs reveal that the only residue near enough to provide stabilization of a positively charged intermediate is an invariant tyrosine (human Tyr-104), which is located above the ribose ring of bound substrates (21, 22, 25). This suggests that these enzymes could provide only minimal electrostatic stabilization for a ribooxocarbenium intermediate formed during an S_n1-type reaction.

An obvious question that arises from the structure of trypanosomal and human HPRTs with PRPP bound (24, 25) is why wasn't the PRPP cleaved. In these structures, PRPP is present in the closed active sites and the metal (M1) is poised to move closer to the O-1 atom to assist further in pulling electrons away from the covalent bond that is to be broken. If S_n1 chemistry were involved, the first half of the reaction might be expected to occur, resulting in dissolution of the covalent bond between PP_i and the ribose monophosphate. However, the reaction seems to be awaiting nucleophilic attack by the purine base, which cannot occur in the reported structures because of the non-reactive purine analog in the active site. Thus, these crystal structures provide evidence for the reaction being associative (S_n2) rather than dissociative (S_n1).

The Design or Discovery of HPRT Inhibitors

Prior to 1999, few HPRT inhibitors had been identified and most yielded K_i values above 10 μM. An exception was a nucleotide analog, carbocyclic GMP, with an IC_{50} of 0.87 μM *versus* a mammalian HPRT (35). Recent progress in enzyme structure-based inhibitor design/discovery shows promise that HPRT inhibitors

eventually might be developed into drugs for the treatment of diseases caused by parasites. In this regard, the *de novo* design of mechanistic inhibitors, including transition state analogs and multisubstrate inhibitors, provides an approach to the discovery of potent inhibitors of HPRT activity (36).

Success in the *de novo* design of a mechanistic type of inhibitor was achieved recently in a series of non-hydrolyzable nucleotide analogs (immucillin 5′-phosphates). As inhibitors of reverse reactions catalyzed by the human HPRT, these compounds yield K_i values in the range of 56–250 nM (37). However, because of "slow onset inhibition," secondary K_i values, determined from the rates of product formation after 2 h in the presence of substrates and inhibitor, were between 1.0 and 14 nM. Although structurally similar to carbocyclic GMP (35), the immucillins differ in that they mimic chemical requirements predicted to exist in the transition state of HPRT-catalyzed reactions (37). Specifically, the immucillins possess nitrogen instead of oxygen in the ring of the ribose moiety and a proton at the N-7 position of the purine moiety. The N-7 proton (predicted to exist for both S_n1- and S_n2-type reaction mechanisms) enables the formation of a hydrogen bond with the invariant Asp-137 residue of HPRTs (21, 22). The nitrogen atom in the ribose ring allows for the formation of a positive charge on the analog of the ribose moiety at physiological pH. In theory, the charge mimics the electrostatic surface potential predicted for a ribooxocarbenium intermediate in S_n1-type reactions. Crystal structures of the human and malarial HPRTs were solved with immucillin inhibitors bound (21, 22). Surprisingly, these structures reveal no strong electrostatic interactions between enzyme residues and the electropositive ribose ring. In explanation, the authors suggest that transition state stabilization is achieved by intramolecular interactions involving the 5′-phosphate of the substrate analog (21). Also, some stabilization could be provided by interactions with the magnesium-coordinated pyrophosphate substrate, whose presence was required to achieve the low K_i values for "slow onset" inhibition (37). Thus, the 2.8-Å hydrogen bond between the N-7 proton of the purine moiety and the invariant aspartate at position 137 (21, 22) as well as altered conformation of the plane of the ribose ring may account for the tight binding characteristics of the inhibitors.

Unfortunately, immucillin 5′-phosphates cannot be tested in their present form as inhibitors of the growth of parasites *in vivo* because they would have difficulty crossing plasma membranes to get to the target enzyme. Also, an intrinsic potential disadvantage of mechanism-based inhibitors is that that they may target all PRTs that have a similar enzyme mechanism, and it may be more difficult to achieve selectivity for binding to the enzymes of pathogens. Thus, a combined approach of mechanism- and structure-based design may be required to achieve target selectivity in mechanism-based inhibitors.

Computational efforts to identify HPRT inhibitors using the x-ray crystal structures of target enzymes have been reported. For example, the rigid body program "DOCK" was used to identify a 240 μM inhibitor of the HPRT from *T. fetus* (38). Derivatives of this lead inhibitor were shown to yield K_i values of 12 μM (38) and 49 nM (39) *versus* the HPRT from *T. fetus*. IC_{50} value determinations with human and tritrichomonal HPRTs indicate that the compounds selectively inhibited the enzyme of the parasite. Inhibition of the growth of parasites *in vitro* by these compounds was reversed by the addition of excess purines in the medium, providing evidence that the HPRT was the most likely target of the inhibitor (38, 39). These are the first reports in which an HPRT inhibitor has been shown to be effective in slowing the growth of a protozoan parasite.

Concluding Remarks

Purine PRTs have been studied extensively during the latter half of the twentieth century. A number of crystal structures have been solved, and considerable data are available from studies of amino acid substitutions that provide insights into structure/function relationships for this important class of metabolic enzymes. Mechanistic studies and enzyme structures have been used in the design and analysis of inhibitors of HPRTs, showing promise that the chemical knock-out of HPRT activity may be achieved within the next few years. Such inhibitors would represent novel leads for the development of drugs for the treatment of diseases caused by protozoan parasites.

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